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In re Patent Application of:
Gregory Gregoriadis, et al.

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For: LIPOSOME-ENTRAPPED DNA ORAL VACCINES

## CLAIM FOR PRIORITY

Commissioner for Patents
Washington, DC 20231

Dear Sir:

Applicant hereby claims priority under 35 U.S.C. 119 based on the following prior foreign application filed in the following foreign country on the date indicated:

| Country | Application No. | Date |
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| European Patent Office | 99307786.6 | October 1, 1999 |

Dated: March 29, 2002

Respectfully submitted,

By _____
Mark J. Thronson
Registration No.: 33,082
DICKSTEIN SHAPIRO MORIN & OSHINSKY LLP
1177 Avenue of the Americas
New York, NY 10036-2714
(212) 835-1400
Attorneys for Applicant

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# Bescheinigung Certificate Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten [illegible] sten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

**Patentanmeldung Nr. Patent application No. Demande de brevet n°**

99307786.6

**PRIORITY DOCUMENT**

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

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Im Auftrag

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# Blatt 2 d r B scheinigung
# Sheet 2 of the certificat
## Page 2 de l'attestation

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Demandeur(s):
Lipoxen Limited
London W1N 3AA
UNITED KINGDOM

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Oral vaccines comprising cationic liposomes and a nucleic acid

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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See for original title of the application
page 1 of the description

# ORAL VACCINES

The present invention relates to oral vaccines comprising cationic liposomes and, complexed or entrapped within the liposomes, a gene vaccine, that is a nucleic acid coding for an antigen against which vaccination is desired.

In WO-A-9810748 gene vaccines are described comprising nucleic acid encoding antigen against which vaccination is required, in which the nucleic acid is entrapped within the liposomes. The liposomes are formed from liposome forming components including cationic lipid. The compositions are said to be suitable for administration by, *inter alia*, oral routes but in the examples, the compositions are administered intramuscularly, subcutaneously, intravenously or intraperitoneally.

For a vaccine to generate an immune response following oral administration, the composition must interact with the lymphoid system in the gut. The vaccine must consequently be stable in the GI tract, and must be stable enough to interact with the relevant cells of the system before being destroyed by bile salts. Clearly it is desirable for vaccines to be administratable orally rather than having to be injected. The present invention relates to compositions which are suitable for oral administration and to oral vaccines and methods for vaccinating human or non human animals by oral administration of the vaccines.

According to a first aspect of the invention there is provided a novel vaccine comprising a nucleic acid operatively encoding an antigen complexed with and/or entrapped within liposomes formed from liposome forming components including

a) at least one cationic compound having the general formula I,

$$R^1OCH_2CH(OR^2)CH_2R^5X^1R^6{}_n \qquad I$$

in which $R^1$ and $R^2$ are the same or different and are selected from groups of the formula $CH_3(CH_2)_a(CH{=}CH\text{-}CH_2)_b(CH_2)_c(CO)_d$-

in which b is 0 to 6, a and c are each selected from 0-23 and (a + c + 3b) is in the range 12-23 and d is 0 or 1;

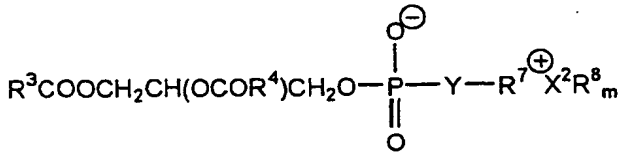
$R^5$ is a bond or a $C_{1-8}$ alkanediyl group a $C_{1-4}$ alkoxy - $C_{1-4}$ alkyl group, or a $C_{1-8}$ oxy-alkylene group ;

$X^1$ is N, P or S;

m is 3 where $X^1$ is N or P and is 2 where $X^1$ is S; and

the groups $R^6$ are the same or different and are selected from hydrogen, $C_{1-8}$ alkyl, $C_{6-12}$ aryl or aralkyl, or two or three of the groups $R^6$ together with $X^1$ may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms;

at least one zwitterionic phospholipid having the general formula II



II

in which $R^3$ and $R^4$ are the same or different and are selected from groups of the formula $CH_3(CH_2)_e(CH=CH-CH_2)_f(CH_2)_g$-

in which f is 0 to 6, each of e and g are 0 to 23 and (e + g + 3f) is in the range 12 to 23;

$R^7$ is a $C_{1-8}$ alkanediyl group;

Y is -O- or a bond;

$X^2$ is N, P or S;

m is 3 when $X^2$ is N or P and is 2 when $X^2$ is S; and

the groups $R^8$ are the same or different and are selected from the group consisting of hydrogen, $C_{1-8}$ alkyl, $C_{6-11}$ aryl or aralkyl, or two or three of the groups $R^8$ together with $X^2$ may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms;

provided that in at least one of the groups $R^1$, $R^2$, $R^3$ and $R^4$, b or f, as the case may be, is 0.

The composition is preferably an oral vaccine and the invention also covers methods of administering the vaccine by oral routes. The composition may comprise pharmaceutically acceptable diluents, and may

include components to enhance the immunogenic properties of the vaccine, such as conventional adjuvants.

In the invention the proviso that at least one of the groups $R^1$, $R^2$, $R^3$ and $R^4$ should have an saturated long chain alkyl group tends to provide a composition which has a relatively high transition temperature. Thus the liposome forming components, in admixture, should have a transition temperature of at least 37°C, preferably in the range 38 to 50°C.

It is preferred that the groups $R^1$ and $R^2$ are the same as one another and that the groups $R^3$ and $R^4$ are the same as one another. In general the present inventors have found that it is desirable that either $R^1$ and $R^2$ are unsaturated and $R^3$ and $R^4$ are saturated, or vice versa. Preferably the cationic compound comprises a single compound of the formula I.

In a particular embodiment of the invention two zwitterionic phospholipids having a different formula, each within formula II, are used in the liposome forming components.

In one embodiment wherein such a mixture is used, in a first zwitterionic phospholipid, the groups $R^3$ and $R^4$ are the same and each represent a group, which f is 1, and in which e + g is in the range 14 to 20, preferably in the range 14 to 18. Preferably the unsaturated group is midway along $R^3$ or $R^4$ that is $e \approx g$, preferably $e = g = 7$. Usually the ethylenic bond is cis.

In a second embodiment wherein a mixture of phospholipids is used in the first phospholipid of a mixture, the groups $R^8$ are preferably all the same and are preferably hydrogen. In the second phospholipid of the formula II, the groups $R^8$ are all the same and are $C_{1-4}$-alkyl. Often in this embodiment, for both phospholipids, f is 0.

Generally, in both embodiments using mixtures of phospholipids in both first and second phospholipids, Y is O and $X^2$ is N. Furthermore $R^7$ is preferably $C_{2-3}$-alkanediyl.

In the cationic compound of the formula I, the hydrophobic groups $R^1$ and $R^2$ may be joined to the rest of the molecule through ether linkages (that is d is 0) or ester linkages (in which d is 1). Preferably in compounds of the

formula I, $R^5$ is $C_{1-4}$-alkanediyl. Preferably the cationic compound is permanently cationic, that is substantially fully ionised at all pH's likely to be encountered *in vivo*, in the range 5 to 9. Preferably each of the group $R^6$ is other than hydrogen, therefore, especially $C_{1-4}$-alkyl, most preferably each group $R^6$ being methyl.

$R^5$ is preferably a bond or a methylene group.

A particularly preferred embodiment of the composition of the invention utilises a cationic compound of the general formula I in which each of the groups $R^1$ and $R^2$ is an oleoyl group and in which the group $R^5$ is a bond, $X^1$ is N and each of the groups $R^6$ is methyl (1, 2-bis(oleoyloxy)-3-(trimethylammonio)propane(DOTAP)). An alternative cationic compound is the analogous compound in the which the hydrophobic oleoyl groups are replaced by oleyl groups i.e. joined through ether linkages rather than ester linkages. A suitable cationic compound in which the hydrophobic groups are saturated is 1, 2-bis(hexadecyloxy)-3-trimethylammino propane(BisHOP).

Suitable zwitterionic phospholipids include dioleoyloxy phosphatidyl ethanolamine (DOPE), dioleoyloxy phosphatidylcholine (DOPC), distearoyl phosphatidyl ethanolamine (DSPE), distearoyloxy phosphatidylcholine (DSPC), dipalmitoyl phosphatidyl ethanolamine (DPPE), dipalmitoyl phosphatidylcholine (DPPC), and admixtures. A particularly preferred zwitterionic phospholipid mixture comprises distearoyl phosphatidylcholine and dioleoyl phosphatidyl ethanolamine.

A mixture of two zwitterionic phospholipids generally comprises the two compounds in weight ratios in the range 10:1 to 1:10, most preferably in the range 5:1 to 1:5, more preferably 2:1 to 1:2. Preferably the proportion of groups $R^3$ and $R^4$ which are saturated in a mixture is at least 50%.

Generally the ratio of cationic compound to zwitterionic phospholipid (total) is in the range 10:1 to 1:20, more preferably in the range 5:1 to 1:10, more preferably in the range 1:1 to 1:5.

According to a further aspect of the invention there is provided an oral vaccine comprising a nucleic acid encoding an antigen complexed to and/or entrapped within liposomes formed from liposome forming components

including at least one cationic compound and at least one zwitterionic phospholipid characterised in that the liposome forming components in combination have a transition temperature of at least 37°C.

The transition temperature is determined by differential scanning calonimetry.

In this aspect of the invention the zwitterionic phospholipids preferably comprise a mixture of lipids, for instance a mixture of saturated and unsaturated lipids, and/or a mixture of phosphatidylcholines and phosphatidylethanolamines.

The cationic compound is preferably a 2,3-di(acyloxy or alkoxy) substituted propylamine derivative, for instance having the general formula I above. Alternatively the compound may be formed of simple cationic amphiphilic compounds such as mono- or di- stearylamine or other long chain alkyl amine, or the secondary, tertiary or quaternary derivatives thereof having, respectively, one, two or three N-lower alkyl ($C_{1-4}$ alkyl) substituents, such as dimethyldioctadecyl ammonium halides. Another category of amphiphilic cationic compounds which are suitable for incorporating into liposomes, is spermine conjugates with di(fatty acyl) glycerides or N,N-di($C_{12-24}$) alkyl acyl amide compounds or DC cholesterol. A range of suitable cationic amphiphilic compounds are described by Kabanov A.V. *et al* in Bioconjugate Chem. (1995), 6(1), 7-20, the content of which is incorporated herein by reference.

According to a further aspect of the invention there is provided an oral vaccine comprising a nucleic acid encoding an antigen complexed to and/or entrapped within liposomes formed from liposome forming components including at least one cationic compound and at least one zwitterionic phospholipid characterised in that the liposome forming components include at least 25 mole%, preferably at least 50 mole%, of components which individually have a transition temperature of more than 40°C.

In this aspect of the invention the effect of using relatively high levels of high transition temperature lipidic components is that the transition temperature of the mixture of liposome for using components will be above

37°C The transition temperature of a mixture tends to be close to the averaged transition temperatures of the individual components. However it is generally easier to determine the transition temperature of individual components, the values for many of these being known. Preferred high transition temperature zwitterionic phospholipids are DPPC ($T_c$ 41.4°C), DSPC ($T_c$ 55.1°C), DPPE ($T_c$ 64°C) and DSPE ($T_c$ 74.2°C).

In all aspects of the invention other components may be included in the liposome forming mixture, such as cholesterol, in amounts up to 50% by weight. Preferably the liposome forming components are free of cholesterol.

The amount of cationic compound is preferably in the range 5 to 50% of the total moles of liposome forming components, preferably in the range 10 to 25% mole.

The liposome composition is generally in the form of an aqueous suspension for instance, a physiological buffer. Alternatively it could be a dried composition for rehydration.

The liposomes may be made by any of the generally used liposome forming techniques. The product liposomes may be multilamellar or unilamellar vesicles and may be relatively large (vesicle diameters in the range 300 nm to 2000 nm preferably with average diameters in the range 500-1000 nm), or small (vesicle diameters in the range 100 nm to 400 nm preferably with average diameters in the range 200 to 300 nm). Preferably the liposomes have a mean diameter not exceeding 1000 nm, and preferably substantially all have diameters less than 2000 nm. Most preferably the mean diameter is in the range 200-750 nm.

In the novel compositions the nucleic acid may be complexed with liposomes that is located externally of the liposomes. Preferably, however, the nucleic acid is at least partially entrapped.

Preferably the liposomes are formed by a process in which the vesicles are formed, mixed with nucleic acid to be entrapped and are then dehydrated, preferably by freeze drying, and subsequently rehydrated in aqueous composition to make dehydration-rehydration vesicles (DRV's), optionally the DRV's may be subsequently subjected to micro fluidization to

reduce the average size. Preferably the non-entrapped material is separated from liposomes by centrifugation or molecular sieve chromatography, after the rehydration and/or microfluidization steps, although this may be unnecessary.

According to a further aspect of the present invention there is provided a method of entrapping polynucleotide into liposomes involving the steps of:

1. forming an aqueous suspension comprising naked polynucleotide which operatively encodes an immunogenic polypeptide useful to induce a desired immune response in a human or animal subject, and preformed liposomes formed of liposome forming components as specified for the novel compositions above,
2. freeze drying or spray drying the suspension, and
3. rehydrating the product of step 2.

Further steps which may be carried out but are not essential are:

4. subjecting the aqueous suspension of dehydration-rehydration vesicles from step 3 to microfluidization to control the size; and
5. optionally separating non entrapped polynucleotide from liposomes. This last step is generally found to be unnecessary, since the external nucleic acid may be partially protected from the environment by being complexed to the cationically charged liposomes.

The dehydration-rehydration of steps are substantially as described by Kirby and Gregoriadis, (1984) Biotechnology, 2, 979-984, the content of which is incorporated herein by reference. Thus, the liposomes in step 1 are preferably small unilamellar (SUV's) (although they may be MLV's for instance having size 2 μm) and made in step 3 are preferably multilamellar liposomes (MLV's) respectively. The product liposomes of step 3 are generally called dehydration-rehydration vesicles (DRV's).

Micro fluidization of the DRV's is carried out substantially as described in WO-A-92/04009, the disclosure of which is incorporated herein by reference and by Gregoriadis et al, (1990), Int. J. Pharm. 65, 235-242.

The present invention does not involve polymerising the liposome forming components to raise the transition temperature. This may reduce the delivery rate of active and is an undesirable extra step in the processing.

By using the DRV technique, inventors have established that up to 90% or even more of the polynucleotide present in the aqueous suspension subjected to the drying step can be entrapped and/or complexed with the liposomes. The level of polynucleotide entrapment and/or complexing in the liposomal composition is preferably in the range 0.05 to 100, preferably 1 to 50, more preferably 5 to 50 µg/µ mole lipid.

The liposome compositions of the invention have been found to be resistant to bile salts and this is believed to correlate with stability in the GI tract.

The nucleic acid active may be RNA, for instance which is directly replicable and translatable in the synthesis of the antigen, or which must first be reverse transcribed to form DNA for replication. Preferably the nucleic acid is DNA which is preferably replicated, and is transcribed and translated to form the antigen of choice. The DNA is preferably a ds plasmid DNA.

The invention includes also the use of the compositions of liposomes or made by the processes of the invention in the manufacture of a composition for use in a method of therapy or prophylaxis. For instance the method may be the immunisation (vaccination) of a human or animal subject to protect it against infection by infectious micro organisms. Alternatively an immune response may be generated by the gene product which is useful in immune therapy, for instance to treat cancer.

The invention is illustrated further in the following examples:

Example 1

Methodology: Oral immunisation experiment 1

**Liposome preparation**

Liposomes with the following compositions were prepared using the Dehydration-Rehydration method (DRV);

1) 32 µmoles of egg phosphatidylcholine (PC), (mixture of di fatty acyl phosphatidylcholines, including some saturated groups)
16 µmoles of dioleoyl phosphatidylethanolamine (DOPE),
[illegible] of dioleoyl trimethylammonium propane (DOTAP)

2) 32 µmoles of distearoyl phosphatidylcholine (DSPC),
16 µmoles of DOPE,
8 µmoles of DOTAP.

3) 32 µmoles of DSPC,
16 µmoles of cholesterol (CHOL),
8 µmoles of DOTAP.

600 µg of pRc/CMV HBS plasmid DNA encoding for the S (small) region of Hepatitis B surface antigen (HBsAg; subtype ayw) was entrapped in the above liposome formulations using the following technique.

The dehydration-rehydration procedure (Kirby and Gregoriadis, (1984) *op. cit.*) was used for the incorporation of pRc/CMV HBS plasmid DNA into liposomes. In short, 2 ml of small unilamellar vesicles (SUV) were prepared from the specified liposome forming components mixed with plasmid DNA and freeze-dried overnight. The liposomes were then subjected to controlled rehydration to generate multilamellar (Gregoriadis et al, (1993) Biochim. Biophys. Acta 1147, 185-193) dehydration-rehydration vesicles (DRV). The product was not subjected to steps to remove non-entrapped DNA and probably includes external DNA complexed to the liposomes.

Entrapment complexation efficiency for each of the compositions was 85-95%.

**Immunisation**

The method is based on Roy, K. *et al* (1999) Nature Medicine 5(4) 387-391.

Groups of 4 female Balb/c mice (20-24g) were immunised orally with either "naked" (group 4) or liposome-entrapped (groups 1-3) DNA using animal feeding needles attached to a 1 ml syringe. Each mouse was fed with 100 µg of DNA in a volume of 500 µl of phosphate buffered saline (PBS) on days 0, 28 and 38.

Mice were bled from groups:

1) PC:DOPE:DOTAP (100 µg DNA) (invention)
2) DSPC:DOPE:DOTAP (100 µg DNA) (invention)
3) DSPC:CHOL:DOTAP (100 µg DNA) (invention)
4) "Naked" DNA (100 µg DNA) (reference)
5) Control (no DNA)

**IgA extraction from foecal pellet**

Foecal pellets were collected from the cages of mice on days 0, 14, 21, 32, 40, 48, 62, 84, 96 and 119.

These pellets were suspended in PBS at a concentration of 100 mg/ml, subjected to centrifugation and the supernatant (containing IgA) was analysed.

**ELISA measurements**

Elisa was done on foecal extracts to measure secretory IgA. Plates were coated with the S (small) region of Hepatitis B surface antigen (HBsAg; subtype ayw), blocked with 1% BSA to avoid nonspecific binding and then pellet extracts added in duplicate (undiluted). Horseradish peroxidase-conjugated goat anti-mouse IgA was added, followed by o-phenylenediamine substrate. Absorbance at 450nm was measured. Results in Figures 1a - i represent mean of duplicate measurements for each group of mice.

Example 2

Methodology: Oral immunisation experiment 2

**Liposome preparation**

Liposomes with the following compositions were prepared using the Dehydration-Rehydration method (DRV):

1) 32 µmoles of DSPC,
16 µmoles of DOPE,
8 µmoles of DOTAP.

2) 32 µmoles of DSPC,
16 µmoles of distearoyl phosphatidylethanolamine (DSPE),
8 µmoles of DOTAP.

3) 32 µmoles of DSPC,
16 µmoles of dipalmitoyl phosphatidylcholine (DPPE),
8 µmoles of DOTAP.

4) 32 µmoles of DSPC,
16 µmoles of DOPE.

pRc/CMV HBS plasmid DNA was entrapped into the above liposome formulations using the same method as Example 1. DRV compositions 1, 2 and 3 entrapped 85 - 95% of the total amount of DNA used. The non-cationic DRV liposomes (composition 4) had an entrapment efficiency of 45-55% (of the total amount of DNA used).

**Immunisation**

Groups of 4 female Balb/c mice (20-24g) were immunised orally with either "naked" (group 6) or liposome-entrapped (groups 1-5) DNA using animal feeding needles attached to a 1 ml syringe. Each mouse was fed with either 50 µg (group 5) or 100 µg (groups 1,2,3,4 and 6) of DNA in a volume of 500 µl of PBS on days 0, 32.

**Immunisation groups:**

1) DSPC:DOPE:DOTAP (100 µg DNA) (invention)
2) DSPC:DSPE:DOTAP (100 µg DNA) (invention)
3) DSPC:DPPE:DOTAP (100 µg DNA) (invention)

4) DSPC:DOPE (100 µg DNA) (reference)

5) DSPC:DOPE:DOTAP (50 µg DNA) (invention)

6) "Naked" DNA (100 µg DNA)

7) Control (no DNA)

**IgA extraction from foecal pellet**

Foecal pellets were collected from the cages of mice on days 0, 42, 55, 65 and 92. These pellets were suspended in PBS at a concentration of 100 mg/ml, subjected to centrifugation and the supernatant (containing IgA)

**ELISA measurements**

Elisa was performed on fecal extracts to measure secretory IgA as for the first oral immunisation experiment. As for the first experiment, results in Figures 2 a-d represent the mean of duplicate measurements for each group of mice.

Conclusions

The conclusions to be drawn from Examples 1 and 2 are that the experiments are repeatable. Furthermore it appears that relatively low levels of entrapped DNA provide adequate transfection rates for an immune response (comparing groups 1 and 5 of Example 2). The saturated lipids seem to produce liposomes having better performance.

## CLAIMS

1. An oral vaccine comprising a nucleic acid operatively encoding an antigen complexed with or entrapped within liposomes formed from liposome forming components including

a) at least one cationic compound having the general formula I,

$$R^1OCH_2CH(OR^2)CH_2R^5X^1R^6{}_n \qquad I$$

in which $R^1$ and $R^2$ are the same or different and are selected from groups of the formula $CH_3(CH_2)_a(CH=CH-CH_2)_b(CH_2)_c(CO)_d$-

3b) is in the range 12-23 and d is 0 or 1;

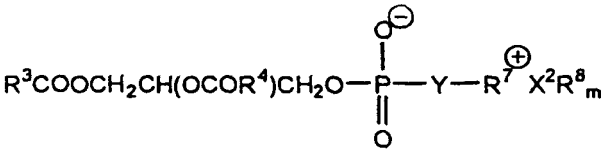
$R^5$ is a bond or a $C_{1-8}$ alkanediyl group;

$X^1$ is N, P or S;

n is 3 where $X^1$ is N or P and is 2 where $X^1$ is S; and

the groups $R^6$ are the same or different and are selected from hydrogen, $C_{1-8}$ alkyl, $C_{6-12}$ aryl or aralkyl, or two or three of the groups $R^6$ together with $X^1$ may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms;

b) at least one zwitteronic phospholipid having the general formula II



$$R^3COOCH_2CH(OCOR^4)CH_2O-P(=O)(O^{\ominus})-Y-R^7X^{\oplus}R^8{}_m \qquad II$$

in which $R^3$ and $R^4$ are the same or different and are selected from groups of the formula $CH_3(CH_2)_e(CH=CH-CH_2)_f(CH_2)_g$-

in which f is 0 to 6, each of e and g are 0 to 23 and e + g + 3f is in the range 12 to 23;

$R^7$ is a $C_{1-8}$ alkanediyl group;

Y is -O- or a bond;

$X^2$ is N, P or S;

m is 3 when $X^2$ is N or P and is 2 when $X^2$ is S; and

the groups $R^8$ are the same or different and are selected from the group consisting of hydrogen, $C_{1-8}$ alkyl, $C_{6-11}$ aryl or aralkyl, or two or three of the groups $R^8$ together with $X^2$ may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms,

provided that in at least one of the groups $R^1$, $R^2$, $R^3$ and $R^4$, b or f, as the case may be, is 0.

2. A vaccine according to claim 1 in which $R^1=R^2$ and $R^3=R^4$.

3. A vaccine according to claim 2 in which $R^1$ and $R^2$ represent a different group to $R^3$ and $R^4$.

4. A vaccine according to claim 2 and claim 3 in which in $R^1$ and $R^2$ b=1 and in which (a + c) is in the range 10-20.

5. A vaccine according to any of claims 2 to 4 in which d = 0.

6. A vaccine according to any of claims 2 to 5 in which f = 0.

7. A vaccine according to any preceding claim in which $X^1$ is N and in which the $R^6$ groups are all $C_{1-4}$ alkyl.

8. A vaccine according to any preceding claim which comprises two zwitterionic phospholipids each having the formula II, in which Y is O, and $X^2$ is N, and the groups $R^8$ of the first phospholipid are all hydrogen and the groups $R^8$ of the second phospholipid and all $C_{1-4}$ alkyl, preferably methyl.

9. A vaccine according to claim 8 in which, in each phospholipid Y is O and $R^7$ is $(CH_2)_h$ in which h is 2 or 3.

10. A vaccine according to claim 8 or claim 9 in which the groups $R^3$ and $R^4$ of the first phospholipid are the same and each is a group in which f=1 and (e + g) is in the range 10 to 20, preferably 12 to 14.

11. A vaccine according to any of claims 8 to 10 in which the groups $R^3$ and $R^4$ of the second phospholipid are the same and each is a group in which f=0 and e + g is in the range 15 to 23, preferably 15-17.

12. An oral vaccine comprising a nucleic acid ncoding an antigen complexed to or entrapped within liposomes formed from liposome forming components including at least one cationic compound and at least one zwitterionic phospholipid characterised in that the liposome forming

components in combination have a transition temperature in the range x to y °C.

13. An oral vaccine comprising a nucleic acid encoding an antigen complexed to and/or entrapped within liposomes formed from liposome forming components including at least one cationic compound and at least one zwitterionic phospholipid characterised in that the liposome forming components include at least 25 mole%, preferably at least 50 mole%, of components which individually have a transition temperature of more than [illegible].

14. A vaccine according to claim 12 or claim 13 in which the zwitterionic phospholipid is selected from the group consisting of distearoylphosphatidylcholine, distearoylphosphatidylethanolamine, diplamitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine and mixtures thereof.

15. A method in which a human or a non-human animal is vaccinated by administering a vaccine according to any preceding claim orally whereby an immune response to the encoded antigen is generated.

16. A method of entrapping polynucleotide into liposomes involving the steps of:

1. forming an aqueous suspension comprising naked polynucleotide, which operatively encodes an immunogenic polypeptide useful to induce a desired immune response in a human or animal subject, and preformed liposomes formed of liposome forming components as defined in claim 1, claim 12 or claim 13,
2. freeze-drying or spray-drying the suspension, and
3. rehydrating the product of step 2.

17. A method according to claim 16 comprising the further steps of:

4. subjecting the aqueous suspension of dehydration rehydration vesicles from step 3 to microfluidization to control their size; and

5. optionally separating non entrapped polynucleotide from liposomes.

## ABSTRACT

## ORAL VACCINES

An oral vaccine comprises liposomes and complexed or, preferably, entrapped DNA operatively encoding an antigen, in which the liposomes are formed from components including cationic compounds and zwitterionic phospholipids. The hydrophobic groups within the liposome forming compounds must include at least one group which is saturated. This is believed to raise the transition temperature, rendering the liposomes more stable when delivered orally. The compositions have been found to give detectable increased in IgA levels, secreted immunoglobulins of importance in efficacious oral vaccine delivery.

Results: Oral immunisation 1

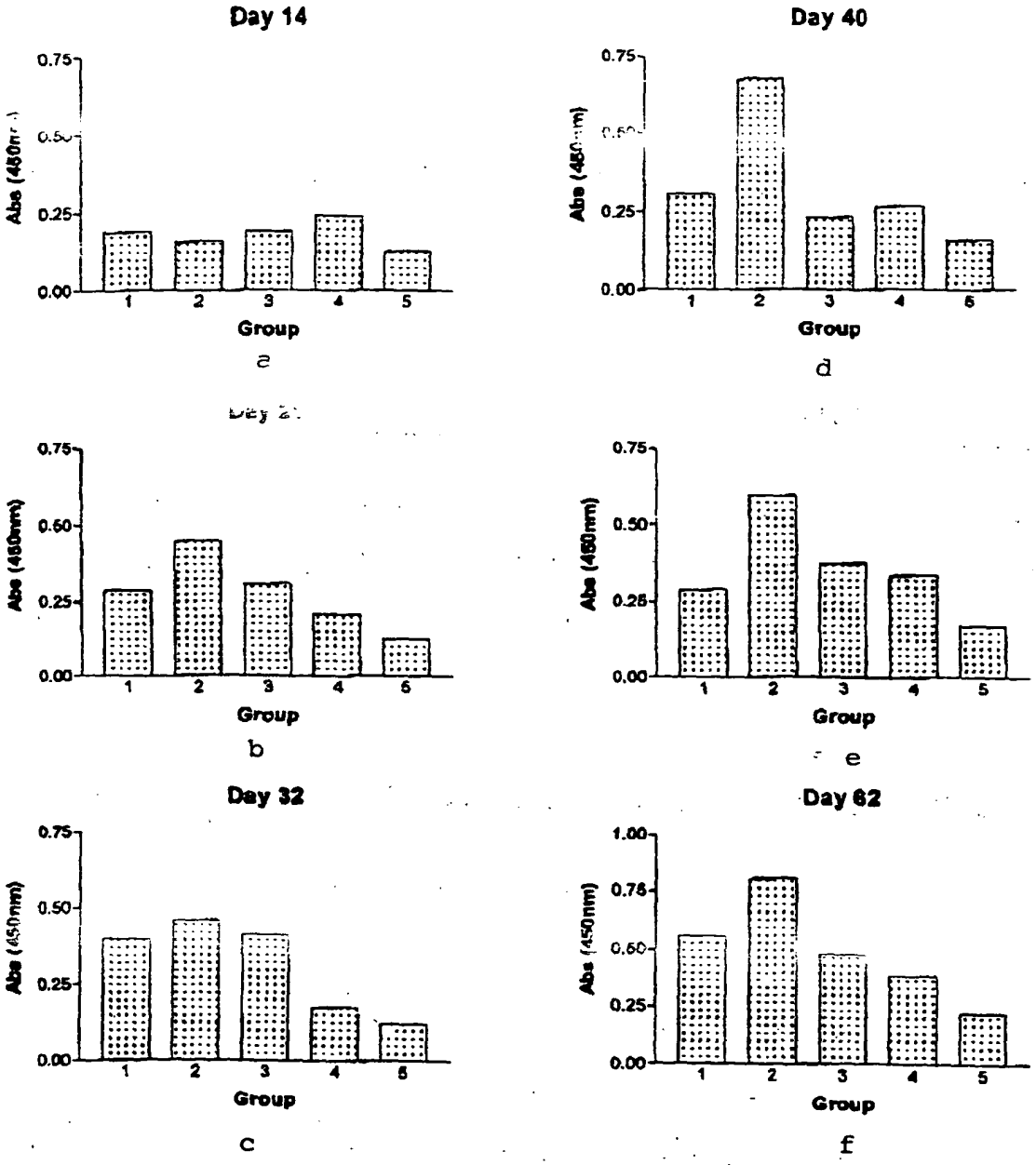
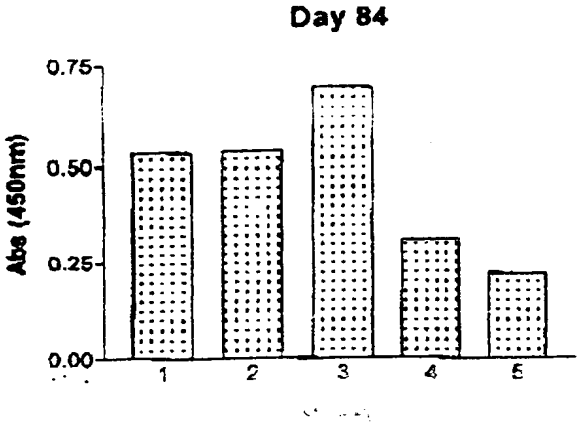
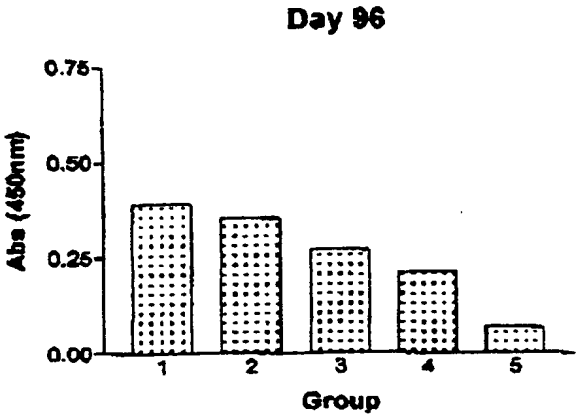



FIG. 1

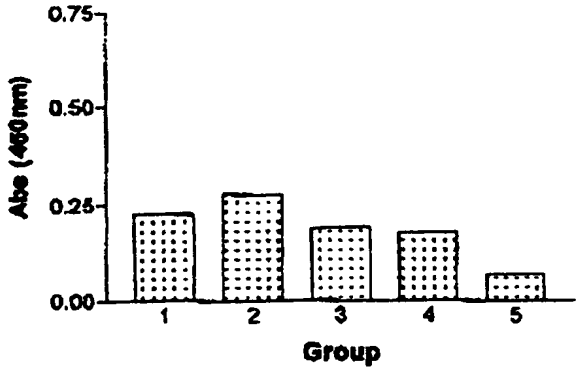
**Results: Oral immunisation 1 (continued)**




g




h




i

FIG. 1 cont...

Results: Oral immunisation experiment 2

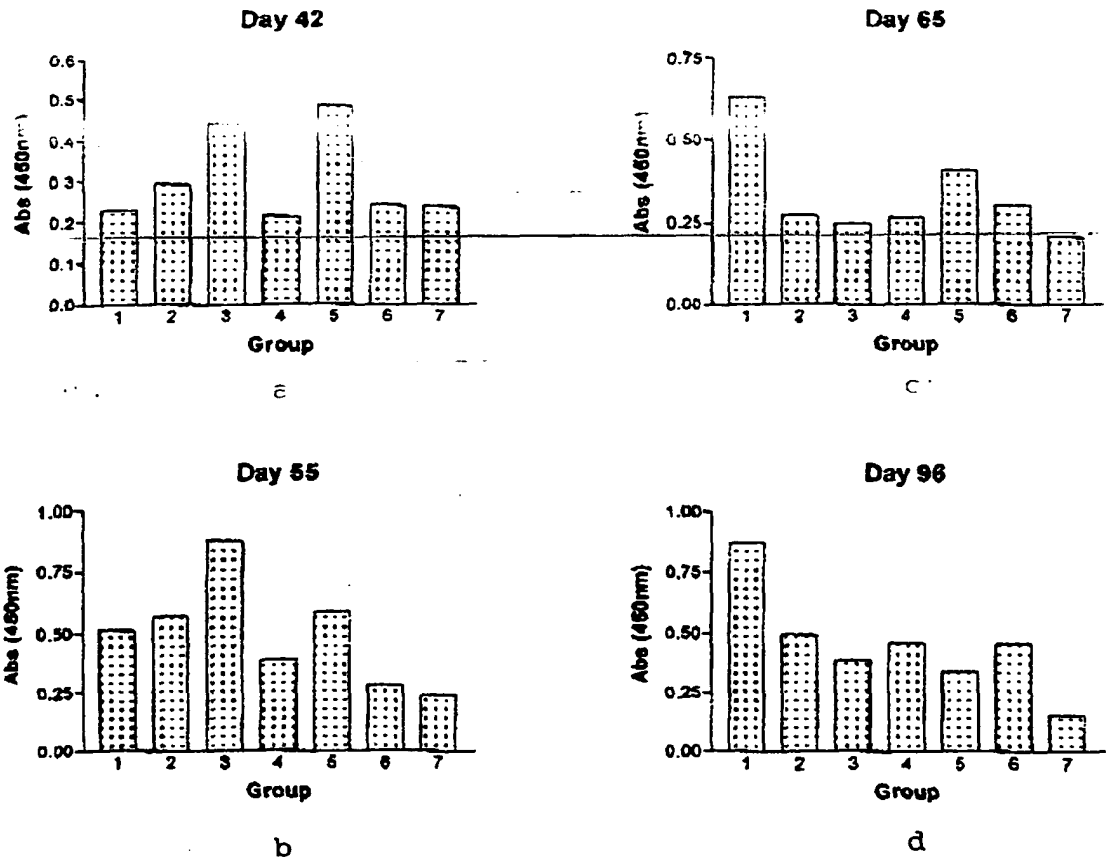



FIG. 2